Original article

Treatment intensification with raltegravir in subjects with sustained HIV-1 viraemia suppression: a randomized 48-week study

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Background: Residual viraemia is a major obstacle to HIV-1 eradication in subjects receiving HAART. The intensification with raltegravir could impact latent reservoirs and might lead to a reduction of plasma HIV-1 viraemia (viral load [VL]), complementary DNA intermediates and immune activation.
Methods: This was a prospective, open-label, randomized study comprising 69 individuals on suppressive HAART randomly assigned 2:1 to add raltegravir during 48 weeks.
Results: Total and integrated HIV-1 DNA, and ultrasensitive VL remained stable despite intensification. There was a significant increase in episomal HIV DNA at weeks 2–4 in the raltegravir group returning to baseline levels at week 48. Median CD4$^+$ T-cell counts increased 124 and 80 cells/μl in the intensified and control groups after 48 weeks (*P*=0.005 and *P*=0.027, respectively), without significant differences between groups. No major changes were observed in activation of CD4$^+$ T-cells. Conversely, raltegravir intensification significantly reduced activation of CD8$^+$ T-cells at week 48 (HLA-DR$^+$CD38$^+$, *P*=0.005), especially in the memory compartment (CD38$^+$ of CD8$^+$CD45RO$^+$, *P*<0.0001). Linear mix models also depicted a larger decrease in CD8$^+$ T-cell activation in the intensification group (*P*=0.036 and *P*=0.010, respectively). Raltegravir intensification was not associated to any particular adverse event.
Conclusions: Intensification of HAART with raltegravir during 48 weeks was safe and associated with a significant decrease in CD8$^+$ T-cell activation, and a transient increase of episomal HIV-1 DNA. However, raltegravir did not significantly contribute to changes in CD4$^+$ T-cell counts, ultrasensitive VL, and total and integrated HIV-1 DNA. These findings suggest that raltegravir impacts residual HIV-1 replication and support new strategies to impair HIV-1 persistence. ClinicalTrials.gov identifier: NCT00554398.

Introduction

HAART results in potent and durable suppression of HIV-1 plasma viraemia in the majority of patients. Although plasma virions and productively infected cells with HIV-1 decay rapidly after HAART initiation, latently infected resting CD4$^+$ T-cells and immune activation decrease very slowly [1–5]. Our current inability to therapeutically eradicate HIV-1, despite prolonged suppression of plasma viraemia measured by standard assays, is ultimately related to the intrinsic stability of the integrated virus genome in resting

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memory CD4+ T-cells [6–8]. However, low-level cryptic replication during suppressive HAART and the potential existence of cellular or anatomical sanctuaries (where antiretroviral drug penetration might be suboptimal) could also play a significant role in a continuous replenishing of the reservoir, making eradication of HIV-1 by HAART even more challenging [9–11].

To date, most treatment intensification strategies in HAART-suppressed subjects have been unable to demonstrate convincing evidence for decay of this latent reservoir [12–17]. However, some preliminary evidences suggest that these approaches can improve antiviral potency and result in a further reduction of residual viral replication [13,18,19]. Paralleling viral burden, chronic immune activation and exhaustion, characteristic of HIV-infected individuals, is not completely normalized in HAART-treated individuals [20] and is widely considered among the best predictors for AIDS progression or response to treatment [20,21]. If residual HIV RNA detectable in plasma and tissues is in part the result of limited antiviral potency and limited rounds of productive viral replication, then antiretroviral therapy intensification might reduce persistent immune activation as well [18]. The intensification of HAART with antiretroviral drugs based on new mechanisms of action could help to test this hypothesis. Raltegravir is a first-in-class integrase strand transfer inhibitor that prevents integration of linear HIV complementary DNA (cDNA) into the host chromosome [22], resulting in a larger representation of non-integrated forms. Among them, circular episomal cDNA has been considered a valid surrogate marker of recent infection [23,24]. We carried out a 48-week randomized study to evaluate the effect of HAART intensification with raltegravir on viral cDNA intermediates and immune activation in HIV-1-infected subjects with sustained virological suppression on standard HAART. We have previously reported 24-week data showing that raltegravir treatment intensification resulted in a transient increase in episomal circular HIV-1 cDNA in a large percentage of individuals [25]. This report presents the complete 48-week study data in the same patient cohort.

Methods

Study design and subjects

This was a prospective, open-label, randomized, Phase III study (ClinicalTrials.gov number NCT00554398) carried out in three university-affiliated hospitals (Hospital Clinic, Hospital Sant Pau and Hospital Germans Trias i Pujol) in Barcelona, Spain. The planned duration was 48 weeks. The institutional ethics review committee at each clinical site approved the protocol, and all participants provided written informed consent.

HIV-1-infected subjects were eligible if they were 18 years of age or older, received a HAART regimen composed of two nucleoside/nucleotide reverse transcriptase inhibitors and a protease inhibitor or a non-nucleoside/nucleotide transcriptase inhibitor, and were naive to integrase inhibitors. Their plasma HIV-1 RNA levels had to be <50 copies/ml at baseline and during at least the previous year. Subjects were randomly assigned 2:1 to add raltegravir (400 mg twice daily) or to continue their HAART. The patient cohort in this study is the same as previously reported for week 24 [25].

Virological and immunological methods

Nucleic acid purification, quantification of circular episomes containing two copies of the HIV-1 long-terminal repeat (2-LTR circles), total and integrated HIV-1 DNA, and plasma single-copy assay techniques were undertaken as described elsewhere [25]. HIV-1 DNA measurements were performed at baseline and at weeks 2, 4 12, 24 and 48. The integrated HIV-1 DNA quantification assay allowed the detection of approximately 40 provirus per million peripheral blood mononuclear cells (PBMCs). The total HIV-1 DNA and 2-LTR circles assays allowed the detection of approximately 1 provirus per million cells. Plasma single-copy determinations were done at baseline and at weeks 12 and 48. Lymphocyte subsets and immune activation analysis were done in fresh blood samples by flow cytometry with two different panels of antibodies to characterize naive or memory subsets, recent thymic emigrants, and the activation of CD4+ and CD8+ T-cells (panel 1: CD3, CD4, CD8, CD31, CD38, CD45RA; panel 2: CD3, CD4, CD8, CD38, CD45RO, HLA-DR) [25].

Statistical analysis

We used the Mann–Whitney U test to compare medians between arms and the signed-rank test (paired test) to compare baseline with week 48. We used linear mixed models to study longitudinal changes within groups and to compare the mean change per week (slope coefficient) between arms. Linear mixed models provide a more powerful means of analysing longitudinal data, including multiple available time points. Since the ultrasensitive viral load (VL) and 2-LTR circles presented either multiple detection limits or a high percentage of values below them, regression order statistics methods were used to calculate summary statistics and comparisons between groups were performed with the Peto–Prentice test. Differences in proportions between groups were analysed through the Pearson's χ^2, considering the continuity correction or the Fisher's exact test, as appropriate. Longitudinal differences in 2-LTR circles and ultrasensitive VL within groups were analysed using

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Table 1. Changes in virological parameters after 48 weeks of intensification with raltegravir

Parameter	Control (*n*=22)	Intensification (*n*=45)	*P*-value between groups
Total HIV-1 DNA			
Median at baseline, copies per million PBMCs (IQR)	14.1 (3.1–61.3)	10.3 (4.5–38.3)	0.713[a]
Median at week 48, copies per million PBMCs (IQR)	54.6 (11.5–367.1)	19.6 (1.4–104.9)	0.043[a]
P-value within group, baseline versus week 48[b]	0.002	0.914	–
Linear mixed models, coefficient (SE)[c]	2.8 (0.49)	-0.42 (0.39)	<0.0001[d]
P-value[e]	<0.0001	0.277	–
Integrated HIV-1 DNA			
Median at baseline, copies per million PBMCs (IQR)	1.9 (0–41.7)	0 (0–7.4)	0.229[a]
Median at week 48, copies per million PBMCs (IQR)	0.4 (0–19.3)	0 (0–3.3)	0.061[a]
P-value within group, baseline versus week 48[b]	0.459	0.406	–
Linear mixed models, coefficient (SE)[c]	0.85 (0.41)	0.09 (0.21)	0.065[d]
P-value[e]	0.039	0.653	–
Ultrasensitive plasma viral load			
Median at baseline, copies/ml (IQR)	0.5 (0.4–0.6)	0.5 (0.4–0.6)	0.334[f]
Median at week 48, copies/ml (IQR)	0.5 (0.2–2.7)	0.4 (0.01–2.8)	0.737[f]
P-value within group, baseline versus week 48[g]	0.782	0.977	–

[a]*P*-value between groups: Mann–Whitney U test. [b]*P*-value within groups: signed-rank test (paired data). [c]Linear mixed model: regression coefficient (mean change per week) and standard error (SE) of the regression coefficient. [d]*P*-value of the interaction term of the linear mixed model: response = intercept + group + week + group × week. A statistically significant interaction term means different slopes in each treatment group. [e]*P*-value of the regression coefficient. [f]Peto–Prentice test. [g]Peto–Prentice–Wilcoxon test. PBMCs, peripheral blood mononuclear cells.

Peto–Prentice–Wilcoxon test for paired data. Statistical analyses were performed with SAS® 9.1 (SAS, Cary, NC, USA) and the R package (The R Foundation for Statistical Computing, Durham, NC, USA).

Results

Participant characteristics

A total of 69 subjects were randomized to intensify their HAART with raltegravir (*n*=45) or to continue their HAART (control group, *n*=24). One subject refused to participate after being assigned to the control group. One subject in the control group met the predefined criteria for virological failure (two consecutive VL>50 copies/ml: 90 and 63 copies/ml at weeks 12 and 24, respectively) and was excluded from the subsequent analysis. Four subjects in the intensified group withdrew consent during the study period (two at week 4, one at week 8 and one at week 12). Two participants in the control group were also prematurely withdrawn due to lost to follow-up (week 12) and for radiotherapy due to a relapsing carcinoma of the uterine cervix (week 36).

Baseline subject characteristics are shown in Additional file 1. All baseline demographic, clinical, virological and immunological ($CD4^+$ T-cell and $CD8^+$ T-cell absolute cells and activation markers) parameters were well-balanced between groups. Subjects in the intensified and control groups had remained with a VL<50 HIV RNA copies/ml prior to randomization for a mean of 5.0 and 4.5 years, respectively (*P*=0.646).

Quantification of total and integrated HIV-1 DNA and 2-LTR HIV-1 circles

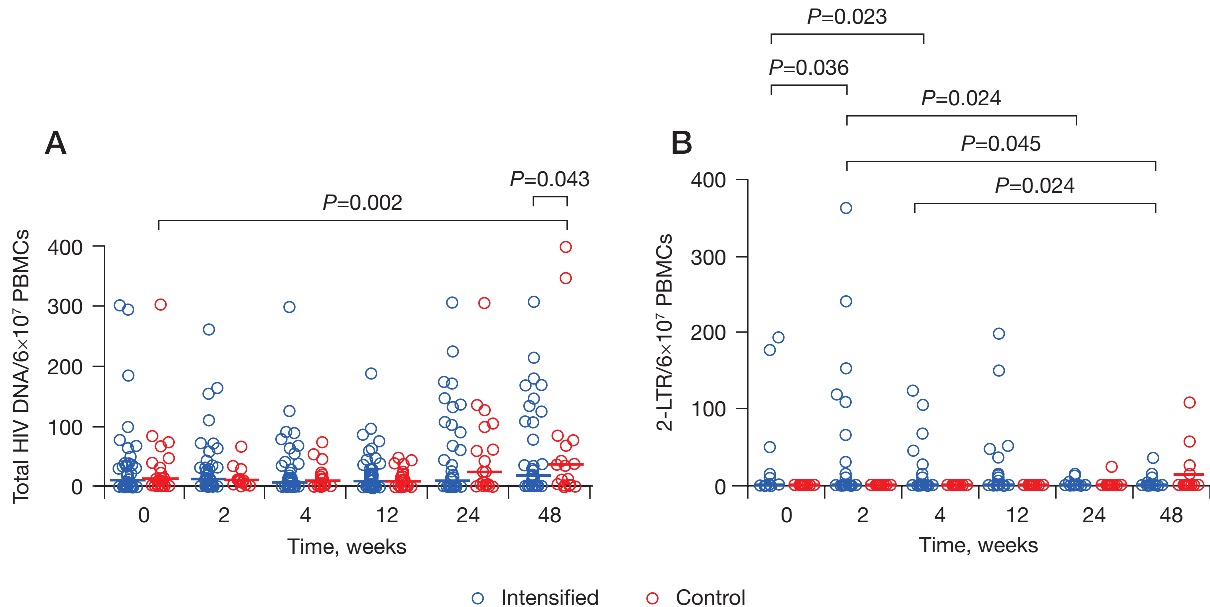
Total and integrated HIV-1 DNA was similar between groups at baseline and did not show any significant changes in the intensified group at week 48 (*P*=0.914 and *P*=0.406, respectively; Table 1). However, the control group showed an increase of total HIV-1 DNA at week 48 compared with baseline (*P*=0.002), resulting in a significant difference between groups at the end of the study (*P*=0.043). The increase of total HIV-1 DNA in the control group was also observed in longitudinal linear mixed model analysis (Table 1). There were no significant differences in integrated HIV-1 DNA between groups at week 48, although the control group displayed a significant slope coefficient in longitudinal linear mixed model analysis (*P*=0.039; Table 1).

There was a significant and transient increase in 2-LTR circles at weeks 2 and 4 compared with baseline in 29% (13/45) of intensified patients, and a significant decrease at week 24, that was sustained at week 48 (Figure 1). No significant differences were found between baseline and week 48 (*P*=0.640).

Ultrasensitive plasma viraemia

The median ultrasensitive plasma viraemia was similar in both groups at baseline and did not show any significant change between or within groups after 48 weeks of drug intensification (Table 1). Overall, ultrasensitive plasma viraemia was detectable in 50% of the subjects at both baseline and week 48, without differences between the intensified (55% and 53% at baseline and

Figure 1. Changes in total and episomal HIV-1 DNA between control and intensified groups at weeks 0, 2, 4, 12, 24 and 48



DNA was extracted from 6×10^7 peripheral blood mononuclear cells (PBMCs) and real-time PCR was used to measure **(A)** total HIV-1 DNA and **(B)** two copies of the HIV-1 long-terminal (2-LTR circles).

week 48, respectively) and the control groups (39% and 45% at baseline and week 48, respectively).

Analysis of lymphocyte subsets and T-cell activation

Absolute CD4$^+$ T-cell counts increased 124 cells/µl in the intensified group after 48 weeks (*P*=0.005) and 80 cells/µl in the control group (*P*=0.027), without significant differences between groups (Table 2). Linear mixed models also showed a significant increase in the CD4$^+$ T-cell count, with slope coefficients of 1.65 and 1.73 cells/µl per week in the control and intensified arms, respectively (Figure 2A and Table 2). Again, comparison of the CD4$^+$ T-cell count mean changes did not show significant differences between groups (*P*=0.902; Figure 2A and Table 2). We further analysed the following CD4$^+$ T-cell subsets: memory (CD4$^+$CD45RA$^-$), recent thymic emigrants (CD4$^+$CD45RA$^+$CD31$^+$) and activated memory (CD4$^+$CD45RA$^-$CD38$^+$ and CD38$^+$ of CD45RA$^-$CD4$^+$). All parameters showed similar values at baseline in both groups and remained stable at week 48 (Table 2). Similarly, the longitudinal analysis using linear mixed models showed no statistically significant differences within and between groups (Table 2). The lack of changes in CD4$^+$ T-cell activation was further confirmed by monitoring the frequency of CD38$^+$HLA-DR$^+$ in CD3$^+$CD8$^-$ T-cells (data not shown). Therefore, the CD4$^+$ T-cell compartment was not specifically affected by the intensification with raltegravir throughout the study period.

Absolute CD8$^+$ T-cell counts increased similarly in both groups at the end of the study (Table 3). Although this change was not statistically significant when comparing baseline and week 48, linear mixed models did show a significant increase in the intensification arm (*P*=0.028). A detailed analysis of the CD8$^+$ T-cell compartment showed that the intensification arm had a reduction of the activation markers measured as CD8$^+$CD45RO$^+$CD38$^+$, CD8$^+$HLA-DR$^+$CD38$^+$ and CD8$^+$HLA-DR$^+$CD45RO$^+$ (Table 3), which was statistically significant for the first two populations (*P*=0.008 and *P*=0.005, respectively). This decrease in activation was particularly relevant in the memory CD8$^+$ T-cell subsets and observed in the frequency of HLA-DR$^+$ (*P*=0.027) and the CD38$^+$ memory cells, the latter measured either by CD38$^+$ of CD8$^+$CD45RO$^+$ (*P*<0.0001) or by an alternative combination of antibodies (CD38$^+$ of CD8$^+$CD45RA$^-$; *P*=0.005).

Longitudinal mixed models depicted in Table 3 showed a decay of CD8$^+$ T-cell activation in the intensification arm, including CD8$^+$CD45RO$^+$CD38$^+$ (*P*=0.0002; Figure 2B), CD8$^+$HLA-DR$^+$CD38$^+$ (*P*=0.0002; Figure 2C), CD8$^+$HLA-DR$^+$CD45RO$^+$ (*P*=0.041), HLA-DR$^+$ of CD8$^+$CD45RO$^+$ (*P*=0.0004), CD38$^+$ of CD8$^+$CD45RO$^+$ (*P*<0.0001; Figure 2D) and

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Table 2. Changes in CD4+ T-cell parameters after 48 weeks of intensification with raltegravir

Parameter	Control (*n*=22)	Intensification (*n*=45)	*P*-value between groups
Median absolute CD4+ T-cell count at baseline, cells/μl (IQR)	503 (371–600)	530 (434–786)	0.333[a]
Median absolute CD4+ T-cell count at week 48, cells/μl (IQR)	583 (420–744)	654 (462–795)	0.381[a]
P-value within group, baseline versus week 48[b]	0.027	0.005	–
Linear mixed models, coefficient (SE)[c]	1.65 (0.44)	1.73 (0.41)	0.902[d]
P-value[e]	0.0003	<0.0001	–
Median CD45RA− at baseline, % of CD4+ (IQR)[f]	65.9 (63.6–74.2)	68.6 (43.0–80.2)	0.943[a]
Median CD45RA− at week 48, % of CD4+ (IQR)[f]	72.1 (57.5–77.4)	68.5 (56.5–78.1)	0.838[a]
P-value within group, baseline versus week 48[b]	0.677	0.608	–
Linear mixed models, coefficient (SE)[c]	-0.337 (0.05)	-0.033 (0.04)	0.273[d]
P-value[e]	0.488	0.354	–
Median CD45RA+CD31+ at baseline, % of CD4+ (IQR)[f]	20.3 (17.2–26.9)	20.3 (12.1–30.7)	0.894[a]
Median CD45RA+CD31+ at week 48, % of CD4+ (IQR)[f]	18.5 (15.7–30.6)	21.1 (14.2–32.0)	0.771[a]
P-value within group (baseline versus week 48)[b]	0.854	0.992	–
Linear mixed models, coefficient (SE)[c]	-0.02 (0.03)	-0.01 (0.03)	0.803[d]
P-value[e]	0.536	0.722	–
Median CD45RA−CD38+ at baseline, % of CD4+ (IQR)[f]	26.8 (23.9–31.7)	26.2 (19.9–35.1)	0.910[a]
Median CD45RA−CD38+ at week 48, % of CD4+ (IQR)[f]	26.1 (23.8–32.2)	25.3 (17.4–34.8)	0.477[a]
P-value within group, baseline versus week 48[b]	0.926	0.082	–
Linear mixed models, coefficient (SE)[c]	0.05 (0.04)	-0.03 (0.03)	0.119[d]
P-value[e]	0.214	0.307	–
Median CD38+ at baseline, % of CD4+CD45RA− (IQR)[f]	37.9 (33.8–47.6)	42.6 (35.3–49.9)	0.587[a]
Median CD38+ at week 48, % of CD4+CD45RA− (IQR)[f]	44.1 (36.6–44.3)	39.1 (33.3–51.0)	0.690[a]
P-value within group, baseline versus week 48[b]	1	0.071	–
Linear mixed models, coefficient (SE)[c]	0.05 (0.05)	-0.03 (0.03)	0.165[d]
P-value[e]	0.272	0.423	–

[a]*P*-value between groups: Mann–Whitney U test. [b]*P*-value within groups: signed-rank test (paired data). [c]Linear mixed model: regression coefficient (mean change per week) and standard error (SE) of the regression coefficient. [d]*P*-value of the interaction term of the linear mixed model: response = intercept + group + week + group × week. A statistically significant interaction term means different slopes in each treatment group. [e]*P*-value of the regression coefficient. [f]Blood samples available for flow cytometry analysis were *n*=34 in the intensification group and *n*=18 in the control group.

CD38+ of CD8+CD45RA− cells (*P*=0.032). Remarkably, the comparison of the mean changes between both arms also showed significant differences in the decrease of CD8+CD45RO+CD38+ (*P*=0.034), CD8+HLA-DR+CD38+ (*P*=0.036) and CD38+ of CD8+CD45RO+ (*P*=0.010; Table 3 and Figure 2). There was no significant association between changes in the activation of CD8+ T-cells and total HIV-1 DNA during the study period. Taken together, these data suggest that long-term treatment intensification with raltegravir specifically reduces CD8+ T-cell activation.

Safety and adverse events

Clinical adverse events of any intensity were reported in 37 subjects (82.2%) in the intensified group and 20 subjects (90.9%) in the control group. Most of them were mild, and most probably unrelated to the antiretroviral regimen. There was one moderate-to-severe clinical adverse event in the intensified group (bacteraemic pneumococcal pneumonia at week 4), compared with 6 in the control group (one pancreatitis at week 8, one anal carcinoma *in situ* at week 12 and [in the same patient] one relapse of a previously known uterine cervical carcinoma requiring surgery and radiotherapy at week 24, one cerebral meningioma requiring craniotomy at week 24, one hypernephroma requiring nephrectomy at week 36 and one ascites at week 48). Although the craniotomy was performed at week 24, the diagnosis of benign meningioma was already known at baseline. There were no grade 3–4 laboratory abnormalities, or significant differences between groups in the evolution of any lipid parameter. Raltegravir was not associated to any particular adverse event.

Discussion

Herein, we describe a significant and transient increase of episomal HIV-1 DNA and a decrease in CD8+ T-cell activation markers over 48 weeks of raltegravir intensification of long-term HAART-suppressed subjects. By contrast, no differences were observed in total and integrated HIV-1 DNA and ultrasensitive plasma VL during the intensification period. This is the largest and longest randomized intensification strategy done so far with raltegravir.

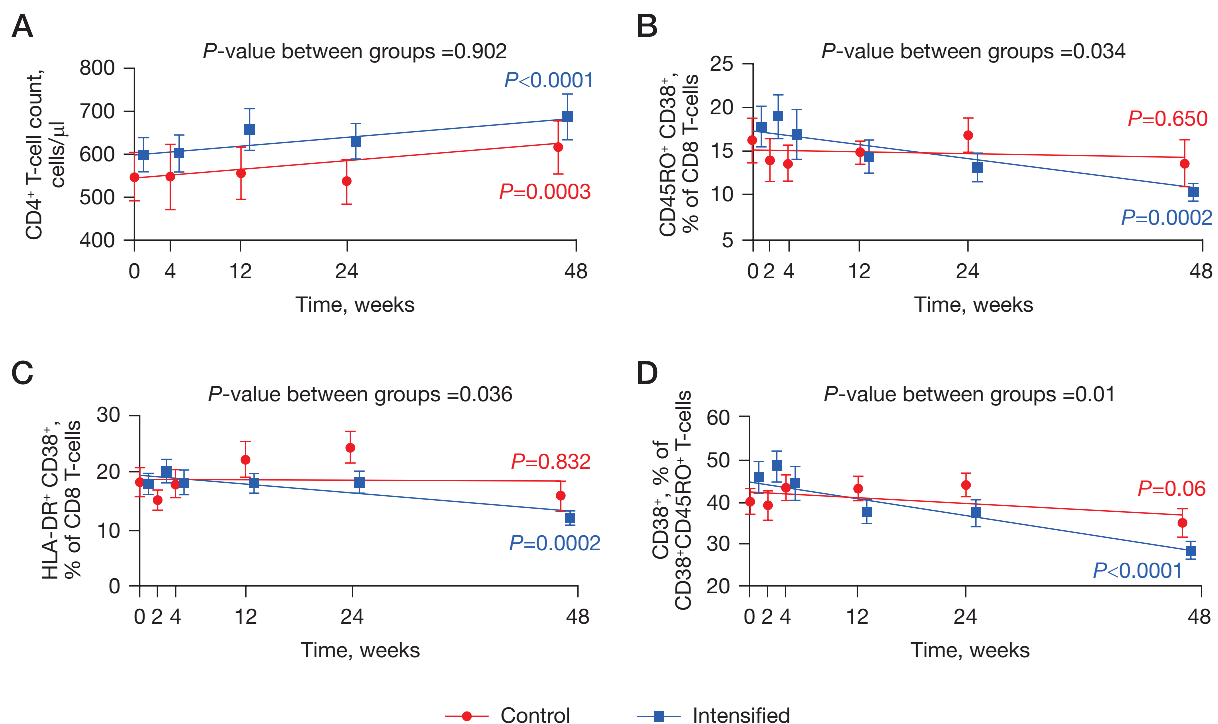
In agreement with the progressive recovery of CD4+ T-cell counts observed beyond 4 years of suppressive

HAART [26,27], both study arms showed significant and similar increases of absolute CD4+ T-cell counts. Of note, a trend towards an increase in absolute CD4+ T-cell counts in the intensification arm was previously reported at week 24 [25], and a similar trend has been described after 12 weeks of intensification with raltegravir in other studies [14]. However, our data points out to no specific impact of long-term treatment intensification with raltegravir in CD4+ T-cell recovery. Consistently, we did not see any specific changes at week 48 in the percentage of CD4+ T-cells, recent thymic emigrants, activated memory CD4+ T-cells and peripheral proliferation of naive CD4+ T-cells.

Immune hyperactivation is a hallmark of HIV-1 infection and plays an important role in the CD4+ T-cell depletion, immune dysfunction and a variety of clinical conditions [28,29]. Different pathways lead to immune activation of T-cells in subjects with HIV-1 infection, which differentially influence CD4+ and CD8+ T-cell subsets. CD8+ T-cell activation is tightly related to viral replication and microbial translocation, whereas CD4+ T-cell turnover would be more dependent on homeostatic forces counteracting CD4+ T-cell depletion [30–32]. Thus, the selective reduction of immune activation in CD8+ T-cells after 48 weeks of intensification is consistent with the hypothesis that raltegravir is blocking residual productive viral replication occurring during apparently suppressive HAART.

In our previous study, a significantly higher level of CD8+ T-cell activation at baseline and a subsequent significant decrease at week 24 were observed among those subjects in the intensification group, with detectable HIV-1 DNA episomes, referred to as the 2-LTR+ subgroup [25]. We now report a significant decrease of CD8+ T-cell activation for the entire raltegravir-intensification group over 48 weeks (Table 2). Of note, both 2LTR+ and 2LTR- subgroups showed significant decreases in CD8+ T-cell activation. Although the 2LTR+ subgroup had a greater decrease, the differences between slopes did not reach statistical significance (data not shown). The improvement seen in the activation of CD8+ T-cell in blood was not unveiled at shorter time points after treatment intensification, suggesting that longer intensification periods are required to significantly reduce immune activation markers. These data are consistent with recently published

Figure 2. Phenotypic analysis of lymphocyte subsets assessed in fresh blood samples by multicolour flow cytometry at weeks 0, 2, 4, 12, 24 and 48



Time course of **(A)** CD4+ T-cell absolute counts, **(B)** CD8+ T-cell activation, measured as **(C)** the percentage of CD8+CD45RO+CD38+ and CD8+HLA-DR+CD38+ cells, and **(D)** the frequency of CD38+ cells in the CD45RO+CD8+ subset. Fitted results from linear mixed models analysis are overlaid on the mean (SEM) of control and intensified groups. *P*-values of longitudinal changes within groups and the mean change per week (slope coefficient) between arms are indicated.

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reports in which raltegravir intensification periods below 24 weeks had not significant effect on CD8$^+$ T-cell activation [14,33]. Although our data suggests a link between residual HIV replication and CD8 T-cell activation, viral coinfections might also contribute to persistent immune activation in HIV-infected individuals; intriguingly, the use of valganciclovir to treat cytomegalovirus infection has shown faster reduction in immune T-cell activation [34].

The effect of raltegravir intensification on HIV burden and T-cell activation in multiple gut sites of seven HIV-positive adults on HAART showed a trend towards decreased T-cell activation in all sites, which was greatest for CD8$^+$ T-cells in the terminal ileum and PBMCs after 12 weeks [11]. Although the study did not reveal a consistent decrease in ultrasensitive plasma viraemia, five of seven participants had a decrease in unspliced HIV RNA per 10^6 CD4$^+$ T-cells in the ileum, suggesting that ongoing viral replication could take place in gut, even if the contribution to plasma RNA is not discernible [11]. Although we did not have access to gut tissue samples from our patients, the early but transient increase in 2-LTR circles in the raltegravir intensified group strongly suggests that recently infected cells might gain access to the peripheral blood from anatomical sites that concentrate HIV DNA and RNA and support persistent productive infection [35]. Cellular redistribution might also explain the transient,

Table 3. Changes in CD8$^+$ T-cell parameters after 48 weeks of intensification with raltegravir

Parameter	Control (*n*=22)	Intensification (*n*=45)	*P*-value between groups
Median absolute CD8$^+$ T-cell count at baseline, cells/μl (IQR)	722 (470–1051)	660 (467–961)	0.631[a]
Median absolute CD8$^+$ T-cell count at week 48, cells/μl (IQR)	850 (613–1070)	716 (488–920)	0.289[a]
P-value within group, baseline versus week 48[b]	0.812	0.095	–
Linear mixed models, coefficient (SE)[c]	0.9 (0.7)	1.1 (0.5)	0.839[d]
P-value[e]	0.198	0.028	–
Median CD45RO$^+$CD38$^+$ at baseline, % of CD8$^+$ (IQR)[f]	14.9 (9.9–18.8)	15.6 (10.7–21.0)	*0.867*[a]
Median CD45RO$^+$CD38$^+$ at week 48, % of CD8$^+$ (IQR)[f]	11.1 (9.1–12.5)	8.5 (6.2–14.0)	*0.146*[a]
P-value within group, baseline versus week 48[b]	0.469	0.008	–
Linear mixed models, coefficient (SE)[c]	-0.02 (0.04)	-0.13 (0.03)	*0.034*[d]
P-value[e]	0.650	0.0002	–
Median HLA-DR$^+$CD38$^+$ at baseline, % of CD8$^+$ (IQR)[f]	16.1 (11.9–21.1)	11.7 (9.4–27.4)	0.690[a]
Median HLA-DR$^+$CD38$^+$ at week 48, % of CD8$^+$ (IQR)[f]	13.3 (7.8–22.6)	9.5 (7.0–15.2)	0.291[a]
P-value within group, baseline versus week 48[b]	0.145	0.005	–
Linear mixed models, coefficient (SE)[c]	-0.009 (0.04)	-0.13 (0.03)	0.036[d]
P-value[e]	0.832	0.0002	–
Median HLA-DR$^+$CD45RO$^+$ at baseline, % of CD8$^+$ (IQR)[f]	19.5 (11.1–25.7)	16.5 (8.9–24.6)	0.502[a]
Median HLA-DR$^+$CD45RO$^+$ at week 48, % of CD8$^+$ (IQR)[f]	10.3 (8.3–17.4)	10.9 (7.7–18.3)	0.771[a]
P-value within group, baseline versus week 48[b]	0.380	0.187	–
Linear mixed models, coefficient (SE)[c]	-0.01 (0.04)	-0.0A7 (0.03)	0.298[d]
P-value[e]	0.800	0.041	–
Median HLA-DR$^+$ at baseline, % of CD8$^+$CD45RO$^+$ (IQR)[f]	45.7 (40.8–59.6)	49.9 (31.6–57.1)	0.814[a]
Median HLA-DR$^+$ at week 48, % of CD8$^+$CD45RO$^+$ (IQR)[f]	35.3 (24.1–52.4)	31.9 (23.4–39.7)	0.293[a]
P-value within group, baseline versus week 48[b]	0.092	0.027	–
Linear mixed models, coefficient (SE)[c]	-0.10 (0.08)	-0.22 (0.06)	0.239[d]
P-value[e]	0.189	0.0004	–
Median CD38$^+$ at baseline, % of CD8$^+$CD45RO$^+$ (IQR)[f]	40.8 (30.4–49.6)	42.5 (32.3–55.8)	0.440[a]
Median CD38$^+$ at week 48, % of CD8$^+$CD45RO$^+$ (IQR)[f]	32.9 (25.5–39.0)	27.0 (19.8–35.9)	0.106[a]
P-value within group, baseline versus week 48[b]	0.266	<0.0001	–
Linear mixed models, coefficient (SE)[c]	-0.11 (0.06)	-0.33 (0.05)	0.01[d]
P-value[e]	0.06	<0.0001	–
Median CD38$^+$ at baseline, % of CD8$^+$CD45RA$^-$ (IQR)[f]	32.1 (24.1–49.2)	34.2 (27.4–49.3)	0.766[a]
Median CD38$^+$ at week 48, % of CD8$^+$CD45RA$^-$ (IQR)[f]	31.9 (27.0–39.5)	30.4 (20.9–40.4)	0.575[a]
P-value within group, baseline versus week 48[b]	0.782	0.005	–
Linear mixed models, coefficient (SE)[c]	0.03 (0.04)	-0.07 (0.03)	0.069[d]
P-value[e]	0.484	0.032	–

[a]*P*-value between groups: Mann–Whitney U test. [b]*P*-value within groups: signed-rank test (paired data). [c]Linear mixed model: regression coefficient (mean change per week) and standard error (SE) of the regression coefficient. [d]*P*-value of the interaction term of the linear mixed model: response = intercept + group + week + group × week. A statistically significant interaction term means different slopes in each treatment group. [e]*P*-value of the regression coefficient. [f]Blood samples available for flow cytometry analysis were *n*=34 in the intensification group and *n*=18 in the control group.

albeit non-significant, increase in CD8 T-cell activation observed at week 2, which may well be related to the complex effect that raltegravir may have in lymphoid organs. Thus, raltegravir might fail to block bystander $CD4^+$ T-cell death in lymphoid tissues, maintaining the associated inflammatory responses [36]. The steady decrease in 2-LTR circles after week 4 and up to week 48 suggests a constant effect of raltegravir on HIV-1 replication, and confirms that frequent sampling early after intensification initiation is required to capture this phenomenon. In the absence of raltegravir, the detection of 2-LTR circles might indicate the existence of sporadic and limited bursts of viral replication.

Despite the evidence for a specific impact of raltegravir intensification in viral and immune dynamics, our study did not disclose any reduction in ultrasensitive plasma viraemia at weeks 12 [25] or 48. Moreover, total or integrated HIV-1 DNA remained stable in the intensified group. In the same way, a short intensification period with raltegravir resulted in no consistent decrease in HIV-1 DNA in the gut in a recent pilot trial [11]. Of interest, we found an unexpected increase in total HIV-1 DNA in the control group with respect to the intensification group after 48 weeks, which was already noticed at week 24 [25]. The reasons for this increase are not associated with changes in $CD8^+$ T-cell activation markers, but might be related to a homeostatic proliferation of latently HIV-1 infected cells in the control group [37]. Although we cannot exclude a transient loss of viral control, all these patients had undetectable VLs (<50 HIV RNA copies/ml) throughout the study, and 12 weeks beyond study completion (data not shown). Moreover, our results might also reflect previously detected variation in levels of the detectable circulating reservoir of HIV DNA in PBMCs from patients with long-term viral suppression [38]. Taken together, these data suggest the limited value of ultrasensitive plasma viraemia and total or integrated HIV-1 DNA to elucidate the benefit of treatment intensification in long-term HAART-suppressed chronically HIV-infected individuals.

The intensification with raltegravir was safe and well-tolerated. An unexpectedly high rate of moderate-to-severe clinical adverse events was seen in the control arm [39], despite their high median $CD4^+$ T-cell count (503 cells/µl) and length of virological suppression. Of note, three subjects suffered neoplasias, although the difference was not significant. Coincidently, these subjects with neoplasia displayed particularly high levels of $CD8^+$ T-cell activation, whose levels have been recently linked to tumour immune control [40–42]. However, the exclusion of these patients from the analysis yielded no differences in the evolution of CD8 T-cell activation markers.

Overall, this study shows that long-term raltegravir intensification is associated with a significant impact in $CD8^+$ T-cell immune activation parameters, as well as a transient increase in HIV-1 2-LTR circles. These findings suggest that HIV-1 replication may persist in some subjects with prolonged virological suppression on standard triple-drug HAART. Moreover, HIV replication probably occurs in anatomical compartments other than peripheral blood, since its blockade has little if any effect on residual plasma viraemia. Direct analysis of tissue samples or, alternatively, a better knowledge of the dynamic exchange of cells and viruses between tissues and circulating blood will help to characterize clinical implications of residual viral replication.

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Additional files

Additional file 1: A table of the baseline characteristics of the participating subjects can be found at http://www.intmedpress.com/uploads/documents/AVT-11-OA-2080_Llibre_Add_file1.pdf

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